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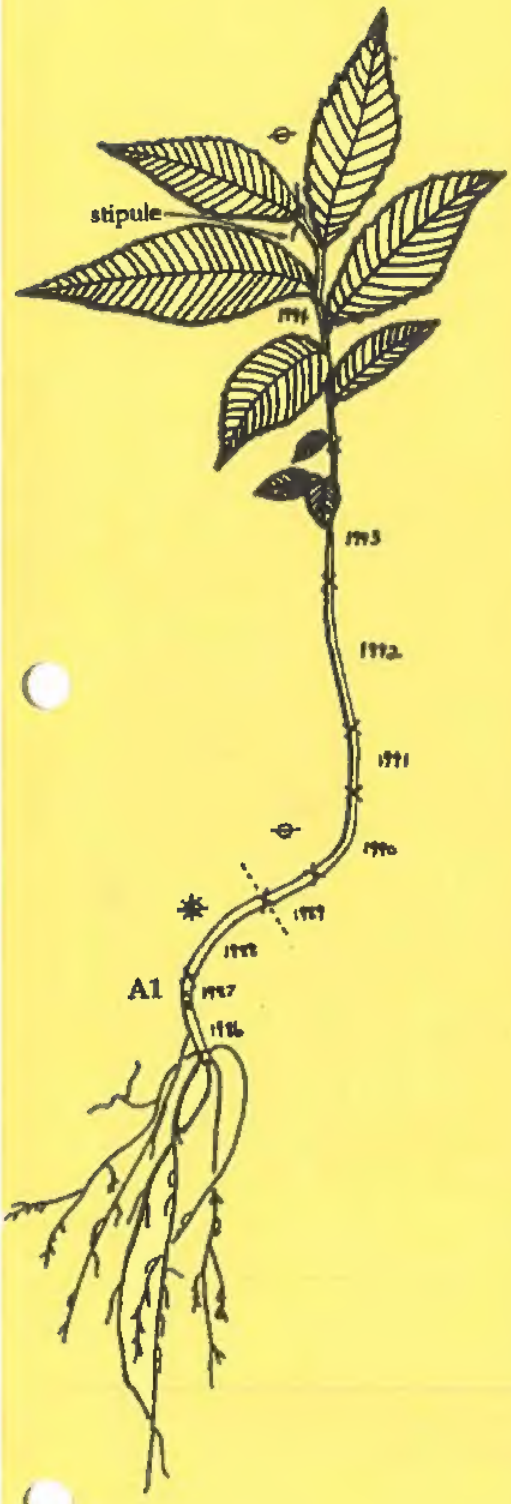


# 32nd PLANT DEVELOPMENT WORKSHOP

## Program and Abstracts

Institut de recherche en biologie végétale
Jardin botanique de Montréal

October 10, 1998

Illustration : *Ulmus americana*. Jeune plant orthotrope âgé de 9 ans.
Tirée de : Jeanne Millet. 1997. *Rapports entre le mode de développement architectural des arbres et le statut successionnel des espèces dans le Québec méridional*. Thèse de doctorat, Université de Montréal.

# 32nd PLANT DEVELOPMENT WORKSHOP

## October 10, 1998

## Institut de recherche en biologie végétale
## Jardin botanique de Montréal

8:30 a. m. **Registration and coffee, Room B354A**

9:15 Welcome - Denis Barabé & Dwight Beebe

SESSIONAL CHAIR : Sylvie Laliberté, UQAM

9:20 **InSun, Kim**
Biology Department, Keimyung University, Taegu 704-701, Korea.
*Ultrastructure of leaves of several Portulaca species.*

9:40 **Petra M. Donnelly, Dario Bonetta, Ronald E. Dengler and Nancy G. Dengler**
Department of Botany, University of Toronto.
*Patterns of cyclin expression in developing leaves of Arabidopsis.*

10:00 **Beebe, Dwight**
IRBV- Université de Montréal
*Specialized differentiation of phloem-cell plasmodesmata associated with initiation of phloem export during post-germination expansion of Cucurbita pepo cotyledons.*

10:20 **Cholewa[1], E., Peterson[1], C.A., Bouchar[2], S., Vonhoff[2], M., Fenton[2], M.B.**
Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.
Department of Biology, York University, Toronto, ON, M3J 1P3.
*The Pathway of Water Supply to Leaves of Tropical Plants Modified by Roosting Bats*

10:40 COFFEE BREAK

11:10 **Millet, Jeanne**
IRBV- Université de Montréal
*Tree architecture and alternance of organization plans.*

11:30 **West, L.J.A. and C.A. Peterson**
Department of Biology, University of Waterloo, Waterloo, Ontario, L2L 1W5.
*Bent Neck Symptom in Cut Rose Stems: Causes and Theories.*

11: 50 **Horton, Roger F**
Department of Botany, University of Guelph
*Inter-tissue signalling, ethylene, and cell separation during abscission.*

12:10 LUNCH

13:10-14 :15 POSTERS (Room B.325)

SESSIONAL CHAIR : Dwight Beebe, IRBV

14:20 **Woodvine, Michelle A. and Nancy G. Dengler**
Department of Botany, University of Toronto, Toronto, ON, M5S 1A1.
*Cellular Correlates of Leaf Expansion in the Heterophyllous Freshwater Buttercup Ranunculus flabellaris.*

14:40 **Peterson, R. Larry & Yukari Uetake.**
Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1.
*Correlated laser scanning confocal microscopy (LSCM) and transmission electron microscopy (TEM) of orchid cells during fungal colonization.*

15 :00 Keynote Talk
**Daniel Matton*, Sylviane Lantin, Marie-Ève Dufresne and Martin O'Brian**
*New approaches to study plant fertilization and embryogenesis.*

15:45 BREAK

16:00 **Vine and Cheese**

# POSTERS

13:10 – 14:15:00

**Barabé, D[1]. and C. Lacroix[2].**
[1]IRBV-Jardin botanique. [2]Department of Biology, University of Prince edward Island.
Morphogenetic gradients and the determination of floral identity in the inflorescences of *Philodendron solimoesense* (Araceae).

**Campeau, Nathalie[1], Andrée Nault[2] and Sylvie Laliberté.**
[1]GREF interuniversitaire, Département des sciences biologiques, Université du Québec à Montréal, QC, H3C 3P8. [2]Biodôme de Montréal, Qc, H1V 1B3.
*Somatic embryogenesis in Panax quinquefolium: effect of seed stratification and developmental stage of zygotic embryos.*

**Dussault, L., M. St-Arnaud, D. Barabé et J.A. Fortin.**
Institut de recherche en biologie végétale, Jardin botanique de Montréal, 4101, rue Sherbrooke Est, Montréal, Qc, Canada, H1X 2B2.
*Effect of Epulorhiza repens on Cypripedium acaule (Orchidaceae) seed germination*

**Komlos, Deborah, John Klironomos and Usher Posluszny**
Department of Botany, University of Guelph.
*Trichome morphogenesis in Salvinia molesta D.S. Mitchell Examined Using Laser Scanning Confocal Microscopy.*

**Kouraichi, Tej[1], Sylvie Laliberté[1] and Denis Barabé[2]**
[1]GREF interuniversitaire, Département des sciences biologiques, Université du Québec à Montréal, Qc, H3C 3P8. [2]Institut de recherche en biologie végétale, Jardin botanique de Montréal
*Variation in the nuclear DNA amount of protocorms of Cypripedium acaule (Orchidaceae) during their in vitro development on a medium with or without dextrose.*

**Lantin, Sylviane and Daniel Matton**
IRBV- Université de Montréal
*Characterization and in situ detection of a developmentally regulated pistil (di)oxygenase.*

**Matsubara, Yoh-Ichi[1], Yukari Uetake[2] & R. Larry Peterson[2]**
[1]Laboratory of Horticulture, Faculty of Agriculture, Gifu University, Gifu 501-1193, JAPAN. [2]Department of Botany, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.
*Early events in colonization of Asparagus officinalis roots by AM fungi.*

**O'Brien, Martin and Daniel Matton**
IRBV, Université de Montréal
*Isolation of pollination and fertilization up- and down-regulated genes through differential display analysis.*

**Poliquin, Julie, and Daniel Matton**
IRBV – Université de Montréal
*Study on plant sexual reproduction : early events following pollination and fertilization.*

**Pravato, Enrico[1-3], Marie-Anne Lelu[2], Mario Houde[3], Catherine Bergounioux[4] and Sylvie Laliberté[1-3].**
[1]GREF interuniversitaire. [3]Département des sciences biologiques, Université du Québec à Montréal, Qc, H3C 3P8. [2]INRA, Centre de Recherches d'Orléans, France. [4]Institut de Biotechnologie des Plantes, Université Paris Sud.
*Changes in the level of a $p34^{cdc2}$ homologue in somatic embryos of hybrid larch, in maturation with or without abscisic acid.*

**Saliba, Iris, Marie Lagacé and Dwight Beebe**
IRBV, Université de Montréal
*Characterization of proteins extracted from Cucurbita pepo isolated cell wall.*

# *ORAL PRESENTATIONS*
(Alphabetical Order)

**Specialized differentiation of phloem-cell plasmodesmata associated with initiation of phloem export during post-germination expansion of Cucurbita pepo cotyledons.**

Dwight U. Beebe. Institut de recherche en biologie végétale, Université de Montréal.

Germinating squash (Cucurbita pepo) cotyledons at different developmental stages were examined by transmission electron microscopy to analyze changes in phloem cell plasmodesmal ultrastructure associated with the initiation of stored assimilate export. In squash cotyledons, bicollateral minor veins are located at the interface between palisade and spongy mesophyll. Minor veins were present in seed cotyledons as procambial strands. Plasmodesmata at the cell-wall interface between precursors of specialized minor vein companion cells (intermediary cells, ICs) and adjacent bundle-sheath cells (BSCs) in both seed cotyledons and during early stages of cotyledon expansion are unbranched and few in number, while numerous branched plasmodesmata are present between BSCs and ICs in minor veins of mature cotyledons. Before a cotyledon undergoes the transition to export, plasmodesmal frequency at this interface increases significantly. These newly formed plasmodesmata were found to be secondary, i.e., they formed across existing walls. Increase in plasmodesmal channel number occurs at least in part, and perhaps entirely, by branching, which results in more channels on the IC-side than on the BSC-side. Thus, secondary plasmodesmata form by the time of the transition to export and may be involved in phloem loading and photoassimilate export.

**Cholewa[1], E., Peterson[1], C. A., Bouchar[1], S., Vonhoff[2], M., Fenton[1], M.B.**
**Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1.**
**Department of Biology, York University, Toronto, ON, M3J 1P3.**

**The Pathway of Water Supply to Leaves of Tropical Plants Modified by Roosting Bats.**

The pathway of water supply to leaves modified into tents by the bats *Ectophylla alba* and *Artibeus watsoni* was investigated. The creation of tents caused extensive damage to the leaf laminas, where up to 50% of the veins were severed. In order to maintain the viability of the leaves, the plant must sustain continuous water transport to the modified areas, and overcome the severe damage caused by roosting bats. Water movement in these leaves was visualized using the red-coloured, water-soluble tracer Safranine O. Detached areas of the leaf lamina were supplied with water *via* minor transverse veins branching from the first major parallel vein that remained intact above the cut. Transverse veins conducted water through a single xylem element and were believed to function only in local water transport, supplying cells in their close vicinity. However, our observations show that transverse veins were able to conduct water and refill severed major parallel veins, keeping the leaf-tent alive for several months. The choice of the roosting under the tents made from the leaves of only certain plant species seems to be adaptive for *E. alba* and *A. watsoni*.

Petra M. Donnelly, Dario Bonetta, Ronald E. Dengler and Nancy G. Dengler.
Department of Botany, University of Toronto. **Patterns of cyclin expression in developing leaves of Arabidopsis.**

Cell cycling plays an important role at several hierarchical levels of plant development, including morphogenesis, cell proliferation and cell differentiation. In this study we use a GUS – cyclin reporter construct to track spatial and temporal patterns of cell division during leaf blade expansion. We show that GUS activity is localized to the cytoplasm of individual cells in dermal, ground and procambial tissues. At the time of leaf blade initiation, the frequency of GUS-expressing cells is high at the incipient leaf margin, but this phase is short-lived and cells at the blade margin cease dividing early. During leaf expansion, the proportion of cells expressing GUS shows a strong longitudinal gradient, with a basiplastic polarity. Adaxial epidermal and palisade mesophyll tissue layers differ in pattern of proliferative cell division: cell cycling within the palisade mesophyll layer is prolonged in comparison to the pavement cells of the adjacent dermal layers. Cell division that is directly associated with cell differentiation, such as the formation of satellite meristemoids and guard cells, is superimposed on the overall pattern of cell division and enlargement in dermal tissue. Our results indicate that cell cycling related to leaf morphogenesis, cell proliferation and cell differentiation overlap during leaf development and that regulatory pathways may require unique features at each hierarchical level.

Roger F Horton. Department of Botany, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

INTER-TISSUE SIGNALLING, ETHYLENE, AND CELL SEPARATION DURING ABSCISSION.

The physical breakage of lignified vascular strands is often the final act in leaf fall. However, vascular strands may be necessary for the initiation of the enzymic hydrolysis of cell walls accompanying cell separation during leaf abscission. Earlier observations and discussions on the requirement and role of vascular tissue in abscission will be reviewed together with data from experiments on the decline in breakstrength and eventual abscission of predetermined stem abscission zones in Crassula argentea. Evidence that ethylene-promoted abscission in these zones has a strong requirement for the presence of vascular tissues will be presented.

**InSun Kim**
**Biology Department, Keimyung University, Taegu 704-701, Korea**
***Ultrastructure of leaves of several Portulaca species***

Morphology, anatomy, and ultrastructure of leaves of several *Portulaca* species were studied. They were divided into two groups based on their foliar morphology. Group A consisted of species with the broad and obovate leaves, while species in group B had linear, lanceolate to terete leaves. Species of group A had paradermal veins and mesophyll (M) cells that completely encircled the veins forming a typical Kranz anatomy. Their bundle sheath (BS) chloroplasts were larger and had well-developed grana and reduced peripheral reticulum (PR). BS mitochondria were larger and more numerous with distinct cristae than those in the M, and chloroplasts in the M cells had well-developed grana and PR. On the other hand, species in group B had peripheral veins and incomplete wreaths of M cells. The chloroplasts in their BS cells were about the same as those in the M cells and had reduced grana but well-developed PR. The BS mitochondria were about the same in size and number as those observed in the M. The M chloroplasts also had well-developed grana and reduced PR. Therefore, the size and structural dimorphism of chloroplast, mitochondria, and microbody were obvious in species of group A, whereas only structural dimorphism of chloroplast was found in group B.

**New approaches to study plant fertilization and embryogenesis**

Daniel P. Matton*, Sylviane Lantin, Marie-Eve Dufresne and Martin O'Brien
Institut de recherche en biologie végétale, Université de Montréal, 4101 Sherbrooke est, Montréal, Québec, H1X 2B2

Plant sexual reproduction is dependant on highly specific interactions between the pollen and the pistil, from the time of pollination, until fertilization and embryogenesis. In order to study early events during fertilization and embryogenesis we are currently isolating genes differentially expressed following these events. The main approach currently used to study embryogenesis at the molecular level is based on the production of mutants, mainly in *Arabidopsis thaliana*. Chemical mutagenesis followed by a map-based cloning strategy or DNA tagging (T-DNA tagging or transposon-tagging) are the most frequently used methods. Although very successful, they are also time-consuming and there seems to be a limitation in the number of genes that can be targeted. Current observations estimate that about only 500 genes readily mutate to give an embryo defective phenotype. Of the estimated 20 000 to 25 000 genes in a typical flowering plant, 20 to 30% are expected to be expressed at some stages during embryo development. This suggest that mutagenesis approaches will uncover only a fraction of the genes expressed during fertilization and embryogenesis. Genes for which there is redundancy either in function and/or in number (gene families) and embryo lethal genes that would act early in embryogenesis will most probably be missed. We are currently undertaking the analysis of both up and down regulated genes following pollination and fertilization in a self-incompatible wild potato species, *Solanum chacoense*. As an alternative to a mutagenesis screen, we have tried various sensitive methods based on differential gene expression. Results from subtractive hybridization, mRNA differential display analysis and virtual subtraction will be presented.

Millet, Jeanne.

Tree architecture and alternance of organization plans.

Architectural analysis was done on nine tree species in the temperate deciduous forest to highlight the link between their developmental pattern and their successional status. The results underline the phenomena of the alternance of hierarchic and polyarchic organization plans during the development of trees. In the hierarchic plan, there is a clear differenciation between the trunk and its branches while in the polyarchic plan, several axes share apical dominance and make a fork (temporary or persistant). The polyarchic organization plan arises with either a spontaneous abortion of the dominant axis apex or a change in its orientation and role. The alternance of the organization plans and the architectural characteristics of trees in each phase of development show, for the nine species, different numbers of organization levels in their architecture. The species that have a higher frequency of alternations of the organization plans in their structure and a higher number of visible organization levels, are the species that are the most shade tolerant and the most dominant in the late stages of forest succession. Conversely, the species with the most hierarchic development and the fewest number of visible organization levels dominate sites receiving most light in the early stages of succession.

PETERSON, R. LARRY & YUKARI UETAKE. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1. **Correlated laser scanning confocal microscopy (LSCM) and transmission electron microscopy (TEM) of orchid cells during fungal colonization.**

Seeds of the terrestrial orchid, *Spiranthes sinensis*, were germinated *in vitro* in the presence of *Ceratobasidium cornigerum*, a known orchid fungal symbiont, and stages in colonization monitored by LSCM and TEM. The overall cytology of embryo and protocorm cells changed as colonization occurred and these changes involved the cytoskeleton, endoplasmic reticulum, vacuoles and mitochondria. Cortical microtubules (MTs) disappeared as fungal hyphae entered embryo and protocorm cells and during peloton formation. MTs were, however, closely associated with the perifungal membrane during all stages of peloton formation and degeneration. Cortical MTs reappeared during peloton degeneration. The cortical array of actin filaments also underwent marked changes during colonization but actin filaments were present during all stages of peloton formation and degeneration. Changes in the positioning of mitochondria and actin filaments during fungal colonization were associated with alterations in the endoplasmic reticulum (ER) membrane system. The ER system was closely associated with pelotons and degenerated fungal hyphae.

West, L.J.A. and C.A. Peterson
Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 1W5

**Bent Neck Symptom in Cut Rose Stems: Causes and Theories**

Drooping of the flower head in cut flowers is due to a lack of water supply to the upper portion of the flower stalk. The stem anatomy below the flower head is comprised of distinct vascular bundles that have few fibres or other supportive cell types. The weight of the flower and the relative weakness of the upper stem region result in wilting of the flower head when turgidity is not maintained. The lack of water supply to the upper stem regions is a result of physical blockages in the xylem tracheary elements. These occlusions can be caused by either bacterial plugs or cavitation of the water column and the formation of emboliems. To fully characterize the stem anatomy, visualize bacterial plugs and detect possible changes in wall chemistry after harvest, histochemical tests were performed on freshly harvested and aged stems. Vessel walls were also viewed by scanning electron microscopy. We are currently working on a method to separate the effects of bacteria and embolisms caused by other factors. To determine the effect of aging in the absence of bacteria, a system is being designed to maintain an aseptic environment throughout the vase-life of the stem. The antibiotic Gentamicin (25 $\mu$g/L) eliminated bacteria from the vase water, although bacterial plugs were still present in a few xylem elements. Once established, the bacteria-free system will be used to compare many cultivars for relative sensitivity to harvest damage.

WOODVINE, MICHELLE A. AND NANCY G. DENGLER. Department of Botany, University of Toronto. Toronto, ON, M5S 1A1. Cellular Correlates of Leaf Expansion in the Heterophyllous Freshwater Buttercup *Ranunculus flabellaris.*

The ternately compound leaves of *Ranunculus flabellaris* exhibit striking environmentally-induced heterophylly, producing short, broad lobes under aerial conditions, and elongate, narrow lobes under aquatic conditions. Abaxial epidermal cells in mature aerial leaves are puzzle-piece shaped while in aquatic leaves, these cells are elongate and rectangular. In this study, we examined cleared leaves that had developed under controlled aerial and aquatic conditions to explore the relationship between: (1) leaf shape as expressed by primary lobe heterophylly index (length:width) and (2) abaxial epidermal cell size, shape and number. Heterophylly indices are significantly different between aerial and aquatic lobes at Day 12, and continue to diverge until leaves cease expansion at Day 32. These differences are correlated with changes in abaxial epidermal cell shape, but not with cell number differences along longitudinal and latitudinal lobe axes. Aquatic leaves exhibit a strong basipetal gradient in cell length between days 12 and 32. In aerial leaves this gradient is not observed to the same extent during expansion of the more isodiametric epidermal cells over the same experimental time period. Smaller-scale variations in cell size are associated with cell position in relation to the base of secondary, tertiary and quaternary lobes. These results demonstrate the tight coupling between whole leaf-based and cell-based developmental events in this heterophyllous species.

# *POSTER*
(Alphabetical Order)

BARABÉ, DENIS[1] and CHRISTIAN LACROIX[2]. [1]Institut de recherche en biologie végétale, Jardin botanique de Montréal, 4101 est, rue Sherbrooke, Montréal, Québec, H1X 2B2. [2]Department of Biology, University of Prince Edward Island, 550 University Ave nue, Charlottetown, Prince Edward Island, C1A 4P3. Morphogenetic gradients and the determination of floral identity in the inflorescences of *Philodendron solimoesense* (Araceae).

A comparative developmental study of the inflorescence of *Philodendron solimoesense* was conducted using scanning electron microscopy. The spadix of *P. solimoesense* is characterized by unisexual flowers. Staminate flowers are initiated on the upper porti on of the spadix while pistillate flowers develop on the lower portion of the spadix. An intermediate zone located between the upper male and lower female portion of the inflorescence consists of flowers that combine features of both sexes. Within this intermediate zone, flowers exhibit polarity with respect to the identity of sexual organs. Stamens are initiated on the flank of the floral meristem facing the upper male zone and carpels are initiated on the portion of the floral meristem facing the low er female zone. The resulting flowers therefore assume a bisexual identity. At the level of the inflorescence, all floral buds are initiated along a series of contact parastichies and the continuity of these parastichies is not disrupted at any level in the male, intermediate, and female zones on the spadix. Results from this study support the presence of a morphogenetic gradient acting at the level of the inflorescence and appears to be independent of the boundaries of floral primordia.

CAMPEAU, NATHALIE[1], ANDRÉE NAULT[2] AND SYLVIE LALIBERTÉ[1]. [1]GREF interuniversitaire, Département des sciences biologiques, Université du Québec à Montréal, Qc, H3C 3P8, [2]Biodôme de Montréal, Qc, H1V 1B3. Somatic embryogenesis in *Panax quinquefolium*: effect of seed stratification and developmental stage of zygotic embryos.

Fresh seeds of American ginseng need an extensive stratification period (18 months; cold-warm-cold) for zygotic embryos (ZE) maturation and germination. We examined the effect of ZE developmental stage and stratification process on somatic embryogenesis (SE) induction. Commercial seeds, harvested in September and kept outdoors in sand for the first cold treatment, were subsequently put in growth chamber at 18°C for 12 weeks, then at 10°C for 8 weeks and 4°C for 15 weeks. Every two weeks during this stratification process, whole ZE were excised, measured and cultivated in the dark at 28°C on MS basal medium with varying growth regulators (GR) and solidifying agents. A distinctly bimodal response was obtained. The first peak of embryogenic activity occurred after the transfer from 18 to 10°C (optimal response on gelrite media without GR: 27% SE). The second, and stronger response followed the transfer to 4°C (50% SE). Reactive ZE were over 2 mm in length, corresponding to the cotyledonary stage. Experiments were also performed using whole ZE and explants (cotyledons, plumule and radicle) from seeds under the 4°C treatment. Cultures were maintained under a 16-hr photoperiod. Up to 70% of the isolated cotyledons cultured on gelrite media initiated SE, as opposed to 54% cultured on agar media, both including 2,4-D and BAP. This response was significantly lower (30.0% and 6.8% respectively) on whole embryos. The ZE developmental stage, the type of explant and the stratification treatment are crucial parameters for optimizing SE induction in American ginseng.

DUSSAULT, L., M. ST-ARNAUD, D. BARABÉ & J.A. FORTIN, Institut de recherche en biologie végétale, Jardin botanique de Montréal, 4101 rue Sherbrooke est, Montréal Qc, Canada H1X 2B2. Effect of Epulorhiza repens on Cypripedium acaule (Orchidaceae) seed germination.

Orchidaceae require a fungal symbiont to grow their protocorm. Cypripedium symbiont has not been isolated and in vitro symbiotic seed germination in this genus was not successful until now. Our objective was to promote symbiotic protocorm growth of Cypripedium acaule, by using 12 fungal strains isolated from pelotons in Orchids species of other genera growing in similar habitats in our latitudes. We have determined oat meal agar (OMA) as the most favourable fungal growth medium, among media having different sources of carbohydrate. Mature seed pods were collected, air dried, and stored at 4° C for two months. Seeds were disinfected in 0.5% sodium hypochlorite, rinsed in sterile distilled water, and sown in two compartment Petri dishes with OMA inoculated with a fungal isolate or uninoculated on one side, and one of four media in the other side to sow the seeds. The media used were a complex medium with dextrose (CA4+), without dextrose (CA4-), OMA or water agar (WA). Regardless of the inoculation treatment, only CA4+ permitted significant seed germination. Among the isolates tested, one Epulorhiza repens strain significantly enhanced the seed germination by comparison with the other isolates and control, although it did not grow in contact with the seeds. Germination level has also been shown to be significantly different between seed pods, indicating different fertility levels among seed pods. We postulate that this E. repens strain produced a signal that enhanced the C. acaule seed germination through the release of a substance or by modifying the chemical composition of the germination medium.

**Trichome Morphogenesis in Salvinia molesta D. S. Mitchell Examined Using Laser Scanning Confocal Microscopy**

Deborah Komlos, John Klironomos, and Usher Posluszny
Department of Botany, University of Guelph

The developmental sequence of trichome initiation and maturation was studied for the aquatic fern, Salvinia molesta D. S. Mitchell (Salviniaceae) with the use of laser scanning confocal microscopy (LSCM). Trichomes in S. molesta originate as protodermal projections, often occurring in pairs, at the margins of young leaves. Outward expansion of the protodermal cells is followed by cell divisions which produce files of several closely-stacked cells. Subsequent expansion of these cells produces elongate trichome hairs. Further toward the midvein, trichomes are initiated in groups of three and four. Concomitant with maturation of the hairs is the outward projection of the papillate structure on which they are borne. On mature leaves, marginal trichomes arise singly and possess a multicellular appearance. Toward the midvein, the tallest trichomes are found, with each comprising four elongate hairs borne on a tall papilla. The final stage in development of the trichomes involves fusion of the four hairs at their tips to produce the characteristic 'egg-beater' morphology of S. molesta trichomes.

KOURAICHI, TEJ[1], SYLVIE LALIBERTÉ[1] AND DENIS BARABÉ[2]. [1]GREF interuniversitaire, Département des sciences biologiques, Université du Québec à Montréal, Qc, H3C 3P8, [2]Institut de recherche en biologie végétale, Jardin botanique de Montréal. Variation in the nuclear DNA amount of protocorms of *Cypripedium acaule* (Orchidaceae) during their *in vitro* development on a medium with or without dextrose.

A morphological study of the *in vitro* germination of *C. acaule* showed the effect of dextrose on the development and the anatomy of protocorms (Leroux *et al.* 1995, Can.J.Bot.). In order to better understand this effect and to correlate morphogenesis with variation in nuclear DNA amount, seeds of *C. acaule* were sowed on a germination medium with (+D) or without (-D) dextrose. Developing protocorms were transferred either i) on the same medium, ii) from +D to -D, or iii) from -D to +D. The relative DNA amount was determined using flow cytometry and the proportion of nuclei in different phases of the cell cycle was evaluated for each developmental stage of the protocorms, before and after transfer to +D and -D medium. During the first developmental stages, in presence of dextrose (+D), the proportion of G1 nuclei was significantly higher, which may suggest the quiescence of cells. After transfer on +D, obovoid protocorms developed to more advanced stages and the proportion of S and G2 nuclei was significantly higher. Moreover, we noted the presence of 4C nuclei, wich may come from the posterior end of protocorms. On -D, protocorms developed to the advanced obovoid stage, where most cells were arrested at G1. After their transfer to +D, we observed a resumption of development and an increase of nuclei in S phase. The higher frequency of S and G2 nuclei during germination, which indicated an active synthesis of DNA, probably reflects cellular events occuring within the anterior end of protocorms.

**Characterization and in situ detection of a developmentally regulated pistil (di)oxygenase.**

Sylviane Lantin* et Daniel P. Matton, Institut de recherche en biologie végétale, Université de Montréal, Montréal, Québec, H1X 2B2.

Plant sexual reproduction is dependant on highly specific interactions between the male gametophyte, the pollen, and the female flower structure, the pistil. Characterization of novel tissue-specific and developmentally regulated genes during pollination and fertilization are currently under way with S. chacoense, a self-incompatible, wild tuber bearing potato. Our current strategy is based both on a subtractive hybridization screen and mRNA differential display. Using the subtractive hybridization technique, we have characterized a developmentally regulated and pollination-induced (di)oxygenase. mRNA localisation as detected by in situ hybridization, shows a developmentally regulated pattern of expression, (di)oxygenase transcripts being detected early on in ovary and style and, as the flower matures, only in the ovary. Pollination increases the amount of (di)oxygenase mRNA, but treatment with various plant hormones do not influence significantly the mRNA levels. This gene is single copy and sequence identities with 2-oxoglutarate/Fe dependant (di)oxygenases, suggests that this protein belongs to this sub-class. The function of this gene will be assayed by plant transformation experiments (both sense overexpression and anti-sense expression) and potential substrates from ovaries will be tested with a His-tagged (di)oxygenase protein expressed in E. coli.

MATSUBARA, YOH-ICHI[1], YUKARI UETAKE[2] & R. LARRY PETERSON[2]
[1]Laboratory of Horticulture, Faculty of Agriculture, Gifu University, Gifu 501-1193, JAPAN. [2]Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1, CANADA. **Early events in colonization of *Asparagus officinalis* roots by AM fungi.**

*Asparagus officinalis* roots have a dimorphic exodermis consisting of short and long cells. Short cells (passage cells) undergo suberization at a slower rate than adjacent long cells and are the preferential entry sites for AM fungi. At a stage at which long cells have developed suberin lamellae and are vacuolated, short cells have a thickened outer tangential wall, lack suberin lamellae and are densely cytoplasmic. Cortical microtubules are well developed in short cells. As hyphae enter these cells and form coils, cortical microtubules undergo changes and a new population of microtubules is associated with the coils. Similar events occur in cortical cells as hyphal coils develop. Cortical cells with developing arbuscules also show changes in microtubules; many microtubules are present around the fine arbuscular branches. As arbuscules degenerate, microtubules are located along the surface of collapsed hyphae and cortical microtubules become more numerous. Uncolonized cortical and vascular parenchyma cells have a well-developed system of cortical microtubules.

**Isolation of pollination and fertilization up- and down-regulated genes through differential display analysis.**

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The objective of our research is to understand the mechanisms of plant sexual reproduction with an emphasis on the molecular genetics of pollen/pistil interactions and the postpollination events leading to embryogenesis, fruit development and seed set. Our current strategy to identify pollination-regulated transcripts in the style and early activated genes in the embryo is based on a mRNA differential display (DD) approach. Despite it's huge potential, DD is often difficult to master because of reproducibility and false positive problems. To overcome these difficulties, we have been using total RNA from time-courses of pollinated pistils as our starting material. The very low amounts of reverse transcribed RNA at the beginning of the amplification step, makes the use of time-courses a quantitative method to select those genes which are either gradually up- or down regulated or transiently expressed. Using only 16 arbitrary primers (covering less than 20 % of the genome analyzed) we have isolated 38 up-regulated and 12 down regulated cDNAs, of which 86 % were found to be true positives after RNA gel blot analyses. Preliminary sequence analysis of some of our clones has revealed sequence identities with pistil specific endochitinase, aquaporin (MIP and TIP), Heat shock protein HSP 80, pectin esterase, ribosomal protein L13, a putative RAB protein and proteins with unknown functions.

**Study on plant sexual reproduction: early events following pollination and fertilization.**

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Following compatible pollination, pollen tube growth and the fertilization events bring about observable changes at the ovary level, including ovary growth and differentiation into fruit as well as maturation of ovules into seeds. These modifications are due to the differential expression of specific genes in the pistil. The aim of my research project is to study the signal transduction events that may lead to the expression of genes specific for fertilization. We face the problem by two approaches. First, we proceed with an in gel kinase assay, with different kinase substrates, on protein samples from ovaries or styles harvested 0 to 96 hours after a compatible pollination. This allows us to monitor changes in protein kinase activities in the pistil induced upon pollination and/or fertilization. Using different cellular fractions (soluble or membrane associated proteins) we have characterized different kinase activities which respond to either pollination or fertilization. We also study, by two-dimensionnal gel electrophoresis, the patterns of in vitro translated proteins, from pistils mRNA present from 0 to 96 hours following compatible pollination. As a model system we have chosen Solanum chacoense, a self-incompatible wild relative of the potato, a plant species closely related to other agronomically important species, like potato and tomato.

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Changes in the level of a $p34^{cdc2}$ homologue in somatic embryos of hybrid larch, in maturation with or without abscisic acid.

Cell division and differentiation are important factors in orientation of the developmental pattern in plants. The $p34^{cdc2}$ protein kinase plays a crucial role in control of the cell cycle, through its association with a cyclin (MPF factor), and its level has been shown to vary in relation to proliferation or differentiation state of the cells. In a comparative histological study on somatic embryos of hybrid larch (*Larix* x *leptoeuropaea*) maturing on a medium including (+ABA) or not (-ABA) abscisic acid, Gutmann et al. (1996, J.Exp.Bot. 47: 1905-1917) showed that +ABA embryos had a high cell division rate, wheres growth in -ABA embryos was mainly due to cell expansion. The aim of our project was to determine the level of the $p34^{cdc2}$ protein in +ABA and -ABA embryos, in order to address the relationship between their developmental pattern and expression of the *cdc2* gene. Cultures were followed over a 5-week period. +ABA embryos developed synchronously and were sampled every week. -ABA embryos developed asynchronously and were therefore sampled at various times during the 5-week period; they were classified and pooled on the basis of their morphology. Using $p34^{cdc2}$ specific antibodies, the protein level was measured with Western blot analysis in whole embryos as well as in isolated cotyledons and hypocotyls. We observed different levels of $p34^{cdc2}$, and different patterns in evolution of this level over maturation time, in relation to embryos developmental stage and growth of hypocotyls and cotyledons. In addition, +ABA tissues showed higher levels than -ABA tissues.

# Characterization of proteins extracted from *Cucurbita pepo* isolated cell wall

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The goals of this research are to identify and characterize proteins associated with or comprising the substructure of plasmodesmata (Pd), in order to understand how Pd function in intercellular macromolecular trafficking. A protocol was developed to isolate a purified cell-wall fraction from minor veins of fully expanded *Cucurbita pepo* cotyledons. A combination of physical removal of both the upper and lower epidermis, pectinase digestion of the abraded cotyledons, followed by sonication and a series of differential centrifugations produced an essentially protoplast-free cell-wall fraction. This cell-wall fraction was then subjected to various extraction methods and the resulting extracted proteins were visualized by one-dimensional SDS-PAGE. Extraction with SDS, either alone or as a component of Laemmli sample buffer, yielded a relatively large number of proteins, approximately 20-30 as visualized by silver staining. Extraction with zwitterionic (CHAPS) and nonionic (Trition X-100) detergents yielded a much smaller number of proteins. Interestingly, extraction with control solutions, either $H_2O$ or our sonication buffer, also produced a small number of bands, some of which appeared to be similar to those extracted by the detergents. The SDS-extracted proteins were tested for the presence of glycoproteins. Only a small number of proteins of about 60-70 kDa gave a positive signal, indicating the presence of glycosylated-proteins. The other SDS-extracted proteins were non-glycosylated. Extraction with $CaCl_2$ also produced a relatively small number of proteins, with a few predominant bands. Only a few of the salt-extracted proteins appeared to be similar to the detergent extracted proteins. The results will be discussed in relation to the ongoing structural characterization of *C. pepo* plasmodesmata and their role in intercellular communication.